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Post-drought growth recovery of tea (*Camellia sinensis*) under different techniques and doses of biofertilizer applications

Abstract. Drought stress is a major limiting factor affecting the growth, yield, and quality of tea (*Camellia sinensis*). The present study aimed to analyze the success of post-drought growth recovery of tea in response to different techniques and doses of biofertilizer applications. Field experiments were conducted from January to August 2024 in the experimental garden of the Research Institute for Tea and Cinchona, Gambung Blok A8, Bandung. This work was arranged in a split-plot design, consisting of a main plot with two biofertilizer application techniques (foliar feeding and soil drenching) and subplots with four levels of biofertilizer dosage (control (B_1), 15 L ha^{-1} (B_2), 22.5 L ha^{-1} (B_3), and 30 L ha^{-1} (B_4), with three replications. The results showed a significant interaction of biofertilizer dose and technique on shoot dry weight and plant growth rate of tea. There was an independent effect of biofertilizer dose on leaf area ratio. Applying a biofertilizer dose of 15 L ha^{-1} through soil drenching produced the best plant growth rate and shoot dry weight at the 6th harvest.

Keywords: Biofertilizer · Post-drought · Plant growth analysis · Tea (*Camellia sinensis*)

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Introduction

Tea plants are susceptible to environmental changes; non-ideal environmental conditions can cause plant stress. The impact of drought can be seen visually in tea plantations, which is the morphological response of tea plants to survive. Under drought stress, the growth of roots, stems, and leaves decreases, accompanied by a reduction in the leaf-to-stem ratio, accelerated senescence of mature leaves, and inhibited development of new foliage (Hemati et al., 2022). Environmental factors such as drought stress in tea plants are the main limiting factors that affect the growth, yield, and quality of tea plants (Qian et al., 2018). Climate change caused by global warming has led to prolonged droughts. Tea growth, yield, and quality are closely related to environmental factors such as temperature, rainfall, and soil health, rendering tea plants highly vulnerable to climate change (Omer et al., 2024). Climate change has a significant impact on plants, as photosynthesis depends on temperature, water, and nutrient availability (Dusenge et al., 2019).

Drought conditions caused decreased biomass, decreased plant height, number of roots, leaf stomatal closure, decreased photosynthetic rate, metabolic disturbances, and increased activity of enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) that play an important role in enhancing drought tolerance (He et al., 2020; Hemati et al. 2022). The crucial condition of tea plants after drought requires optimal strategies, such as fertilizers, rainwater harvesting, irrigation systems, etc. Plant management based on ecophysiology, which includes internal and external factors of tea plantations such as plants, climate, and sufficient nutrients, can improve the growth and quality of tea (Anjarsari et al., 2020). According to Kudoyarova et al. (2015), the availability of water and nutrients significantly affects plant growth and productivity. Plant shoots' development depends on the water supply and nutrients in the soil, especially nitrogen and phosphorus.

Fertilization is one of the strategies used to recover tea plants after drought; however, tea plantations generally rely on inorganic fertilizers in the cultivation process. Repeated application of chemical fertilizers will cause damage to soil properties and environmental damage (Gebrewold, 2018). According to Debele (2021), the main factor leading to decreasing soil fertility is the continuous use of inorganic fertilizers, which causes degradation of soil structure and reduces soil aggregation so that nutrients are easily lost through fixation, leaching, and increases soil acidity. Biofertilizers help the growth of tea plants post-drought, where plants are in the production phase, so they need sufficient nutrients to maintain and increase production. The biofertilizer microorganisms used in this research are categorized as nitrogen fixing (*Azospirillum* sp. and *Azotobacter* sp.) and phosphate solubilizing (*Bacillus* sp. and *Pseudomonas* sp.). Microorganisms in biofertilizers can provide nutrients and promote plant growth under stressful conditions by solubilizing phosphate, fixing nitrogen, and providing other macro or micronutrients (Singh et al., 2022).

The previous study by Reetz et al. (2015) reported that efficient and effective fertilization can be obtained by paying attention to the type of fertilizer, fertilizer dosage, fertilizer application time and technique. According to Niu et al. (2020), the application of fertilizer through the leaves allows plants to absorb nutrients directly through the leaves (stomata), while fertilization through the soil is absorbed by the plant's roots. Application of biofertilizers in excessive doses cause competition between microbes for nutrients, and the performance of biofertilizers is less effective if the application of excessive or insufficient doses (Sinulingga et al., 2015). Rhizosphere microorganisms get energy sources from root exudates, while phyllosphere microorganisms get energy sources from carbohydrates released by leaves (Ali et al., 2024; Bashir et al., 2022).

After drought, plants experience physiological disorders due to a lack of water, nutrients, and oxidative damage. Applying biofertilizers at optimal doses combined with proper fertilization techniques can support tea plant growth by fixing nitrogen, dissolving phosphate, increasing nutrients, and phytohormones (gibberellin, cytokinin, auxin). The effectiveness of fertilizer dosage is determined not only by the amount applied but also by the technique of application, which affects the efficiency of nutrient uptake by the plant. Lower doses may be able to provide optimal results under proper fertilization techniques. The decline in tea productivity due to drought requires appropriate recovery strategies. The

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application of biofertilizers with proper fertilizer application techniques has the potential to enhance nutrient uptake efficiency and accelerate plant recovery. This study aims to evaluate biofertilizers' effectiveness and determine the optimal fertilizer application technique and dosage to support tea plants' growth and leaf yield after drought stress.

Materials and Methods

Field experiments were conducted from January to August 2024 in the experimental field of the Research Center for Tea and Chincona, Gambung block A8, Bandung district. The experimental field is 1,350 meters above sea level (MASL) with Andisol soil order. The average rainfall is 2,960 mm year^{-1}. The materials used in this research include: 1) 27-year-old tea plant clone GMB-7, 2) liquid biofertilizer containing nitrogen-fixing microorganisms *Azospirillum* sp. and *Azotobacter* sp. (1.24 x 10^{11} CFU/ml), and phosphate-solubilizing microorganisms *Bacillus* sp. and *Pseudomonas* sp (1.36 x 10^{11} CFU/ml), 3) Inorganic fertilizers (Urea, TSP, KCl, and Kieserit). The tools used in the research are hand sprayer for biofertilizer application, measuring cup, bamboo, plastic clips, measuring type Krisbow 7.5 m, hoe, tea harvesting net, permanent marker, analytical scales, and scanner Canonscan Lide 200.

The experimental design used a split-plot design, consisting of a main plot with two levels of biofertilizer application techniques and a subplot with four levels of biofertilizer dosage, with three replications. The main plot consisted of foliar feeding (A_1) and soil drenching (A_2). In contrast, the subplots consisted of different dosage treatments: without bio-fertilizer (B_1), 15 L ha^{-1} (B_2), 22.5 L ha^{-1} (B_3), and 30 L ha^{-1} (B_4), which were based on the recommended application doses for tea plants and represent decreasing levels to evaluate the optimal dosage for enhancing post-drought recovery. Liquid biofertilizer was dissolved in 500 ml of water according to the calibration of tools in the field, applied according to the treatment, namely without biofertilizer (500 ml of water), 15 L ha^{-1} (13.5 ml/plant + 486.5 ml of water), 22.5 L ha^{-1} (20.25 ml/plant + 479.75 water), and 30 L ha^{-1} (27 ml/plant + 473 ml of water). The biofertilizer was applied a day after the pre-plucking, with an application interval of 25 days, 6 times. The biofertilizer application technique was carried out according to the treatment, namely, sprayed on the leaves evenly using a hand sprayer and drenched into the soil using a measuring cup. Each experimental unit measured 2 x 5 m with 10 plants, totaling 24 units. A row of tea plants was used as a border between plots.

The plucking is done using plucking scissors. The type of medium plucking is shoots consisting of pecco shoots with two leaves (p+2), three young leaves (p+3m), or dormant shoots with two or three young leaves (b+2m, b+3m). The observation parameters included leaf area, shoot dry weight, leaf area ratio, and plant growth rate. Leaf area and shoot dry weight were obtained from 50 g of fresh shoots taken from each plucking. Plucking was conducted every 25 days over six sampling periods (Sepriana et al., 2023). After sampling, leaf area was measured using an image scanner and ImageJ software.

Leaf area ratio and plant growth rate were calculated using the formula according to (Pandey et al., 2017). The data from the analysis and observations were processed and statistically analyzed using the SmartstatXL program. The data were organized into an analysis of variance (ANOVA) table and analyzed using the F-test (Fisher) at a 95% confidence level. If the analysis of variance indicated significant differences, the results were further tested using Duncan's Multiple Range Test at the 5% significance level.

1. Crop Growth Rate (CGR): The increase in plant dry matter production per unit area per unit time was measured at 25-day intervals over six sample periods.

$$CGR = \frac{W_2 - W_1}{GA(t_2 - t_1)} \text{ g m}^{-2} \text{ day}^{-1}$$

Where:
W = shoot dry weight
t = time
GA = Ground Area

2. Leaf Area Ratio (LAR): the ratio of leaf area to dry weight, which indicates the efficiency of leaf surface in producing dry matter, was measured at 25-day intervals over six sampling periods.

$$LAR = A/W \quad \text{cm}^2 \text{ g}^{-1}$$

Where:
A = leaf area
W = shoot dry weight

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Results and Discussion

Leaf Area. Leaves are the main organs of plants where photosynthesis occurs. According to Huang et al. (2019), plant leaves are where light energy is processed into chemical energy and carbohydrates (glucose) through photosynthesis. According to Tondjo et al. (2015), plant leaves play a role in the formation of biomass through the process of photosynthesis. In tea production, the apical shoots along with the first two to three leaves are regularly harvested continuously, so the tea leaf area is an important component in studying tea plant physiology (Jayasinghe et al., 2015). Figure 1 shows the leaf area of tea plants for 6 times of pluckings. Different fertilizer application techniques did not significantly affect the leaf area of tea plants in a short period. Post-drought tea plants have a plucking layer thickness that is less than optimum (5-10 cm), implying that plant lacks assimilate to expand the leaves. The ideal plucking layer thickness is around 15-20 cm (Anjarsari et al., 2021). Leaf expansion requires an adequate supply of carbohydrates from source organs, mainly from mature leaves (Costa et al., 2007). The photosynthetic capacity of young tea leaves, young buds, and shoots depends on the layer of maintenance foliage to supply the assimilates (Hajiboland, 2017).

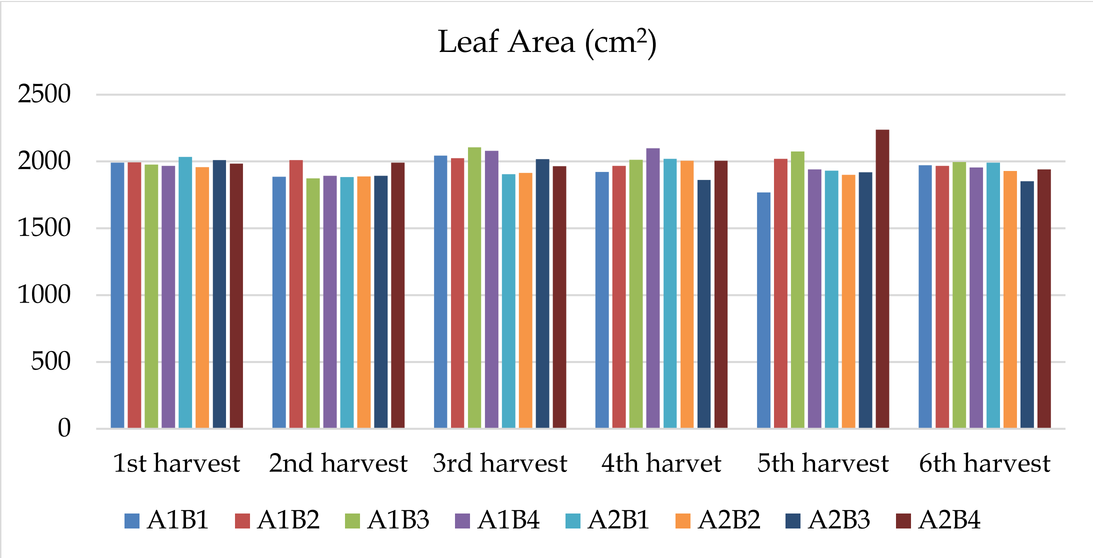

Figure 1. Leaf area (cm²) of tea plants for 6 times of harvests in response to different biofertilizer application techniques and doses

Note: foliar feeding + without bio-fertilizer (A1B1), 15 L ha^{-1} via foliar feeding (A1B2), 22.5 L ha^{-1} via foliar feeding (A1B3), 30 L ha^{-1} via foliar feeding (A1B4), soil drenching + without bio-fertilizer (A2B1), 15 L ha^{-1} via soil drenching (A2B2), 22.5 L ha^{-1} via soil drenching (A2B3), 30 L ha^{-1} via soil drenching (A2B4).

Table 1. Interaction effect of biofertilizer application techniques and doses on shoot dry weight (g) at 2nd harvest.

Treatment	Biofertilizer Doses (L ha^{-1})			
	B_1 : 0 L	B_2 : 15 L ha^{-1}	B_3 : 22.5 L ha^{-1}	B_4 : 30 L ha^{-1}
A_1 : Foliar feeding	12.67 a B	13.77 a A	12.97 a AB	13.10 a AB
A_2 : Soil drenching	12.81 a AB	12.31 b B	12.93 a AB	13.35 a A

Note: The mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level. Lowercase letters (a,b) read vertically, comparing the two techniques at the same dose (within a column). Uppercase letters (A, B) read horizontally, comparing four doses for the same technique (within a row).

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Shoot dry weight. There was an interaction effect between biofertilizer application technique and dose on shoot dry weight at the 2nd, 5th, and 6th plucking. At the first plucking, the application of biofertilizer with various fertilization techniques did not show any significant effect on shoot dry weight. Meanwhile, during the 3rd and 4th pluckings, the decrease in rainfall likely influenced the plant's response to fertilizer application, thereby affecting the resulting shoot dry weight. In May and June, the rainfall decreased to 128.3 mm/month and 220.4 mm/month, respectively.

Table 1 shows the interaction effect between biofertilizer application technique and dose at the 2nd harvest. The treatment sprayed on the leaves at a dose of 15 L ha^{-1} was able to produce the highest dry weight, while application through the soil requires a higher dose of 30 L. This is probably because nutrients are absorbed more quickly through the leaves. Dry weight shows that plants get enough nutrients, because bacteria in biofertilizers help nitrogen fixation and phosphate solubilization. This indicates that plants get enough nutrients. According to Anjarsari et al. (2021), the dry weight of tea shoots reflects the net accumulation of CO_2 in a unit of time based on the plucking cycle, where the increase in dry matter becomes the main parameter in quantitative analysis of plant growth. According to Niu et al. (2020), fertilizer application through the leaves accelerates nutrient absorption, and nutrients enter through the stomata. Fertilizer application through the leaves is absorbed quickly through the pores and leaf surface, so nutrients are absorbed faster than through the roots (Gupta et al., 2023).

Table 2 shows an interaction effect of biofertilizer application doses and techniques on shoot dry weight at the 5th harvest. The treatment of soil drenching at a dose of 30 L ha^{-1} gives the best results, while in foliar application, the dose levels showed relatively similar values. When applied to the soil, microbes need time to adapt and colonize, so the application of fertilizer to the soil requires a longer time to be able to increase the dry weight of shoots. According to Demir et al. (2023), when biofertilizers are applied to the soil, microorganisms will colonize the rhizosphere and help increase the efficiency of plant nutrient absorption. According to Fageria et al. (2009) plants respond to fertilizers applied through the soil for a longer time than foliar fertilization. Nutrients applied through the soil have a long-term effect on plant growth, while foliar application is only temporary. Rainfall aids nutrient dissolving in the soil and can also affect the performance of foliar-applied biofertilizers. The 5th plucking was done in July with 320 mm/month of rainfall. Rainfall has a negative effect on foliar application. Strong winds and rain can directly affect bacterial colonization on leaf surfaces (Bashir et al., 2022).

Table 3 shows an interaction effect of biofertilizer application doses and techniques on the dry weight of shoots at the 6th harvest. The biofertilizer treatment sprayed onto the leaves at a dose of 22.5 L ha^{-1} gives the best results, while application through soil showed relatively similar values at the different dose levels. 6th plucking was conducted in August, where the experimental field rainfall was below optimal at 79.1 mm/month, so the biofertilizer application technique would affect the effectiveness of nutrient absorption. According to Supriadi & Rokhmah (2014), optimal rainfall for tea plant growth ranges from 223-417 mm/month. According to Mandic et al. (2015), foliar application of nutrients can reduce the impact of abiotic stress caused by low rainfall.

Table 2. Interaction effect of biofertilizer application techniques and doses on shoot dry weight (g) at 5th harvest.

Treatment	Biofertilizer Doses (L ha^{-1})			
	B_1 : 0 L	B_2 : 15 L ha^{-1}	B_3 : 22.5 L ha^{-1}	B_4 : 30 L ha^{-1}
A_1 : Foliar feeding	15.11 a	16.14 a	16.53 a	15.66 b
	A	A	A	A
A_2 : Soil drenching	15.12 a	14.07 b	15.07 a	17.54 a
	B	B	B	A

Note: The mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level. Lowercase letters (a,b) read vertically, compare two techniques at the same dose (within a column). Uppercase letters (A, B) read horizontally, compare four doses for the same technique (within a row).

Table 3. Interaction effect of biofertilizer application techniques and doses on shoot dry weight (g) at 6th harvest.

Treatment	Biofertilizer Doses (L ha^{-1})			
	B_1 : 0 L	B_2 : 15 L ha^{-1}	B_3 : 22.5 L ha^{-1}	B_4 : 30 L ha^{-1}
A_1 : Foliar feeding	14.78 a	16.42 a	17.80 a	17.29 a
	C	B	A	AB
A_2 : Soil drenching	15.19 a	16.20 a	15.14 b	16.19 a
	A	A	A	A

Note: The mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level. Lowercase letters (a,b) read vertically, comparing between two techniques at the same dose (within a column). Uppercase letters (A, B) read horizontally, comparing four doses for the same technique (within a row).

Table 4. Independent effect of biofertilizer application techniques and doses on leaf area ratio (cm^2 g^{-1}) at 6th Harvest

Treatment	Leaf Area Ratio (cm^2 g^{-1})
Biofertilizer application technique	
A_1 : Foliar feeding	119.69 a
A_2 : Soil drenching	123.53 a
Biofertilizer application dose (L ha^{-1})	
B_1 : 0 L	132.36 a
B_2 : 15 L ha^{-1}	120.34 b
B_3 : 22.5 L ha^{-1}	117.14 b
B_4 : 30 L ha^{-1}	116.61 b

Notes: The mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level.

Many foliar microorganisms are capable of producing extracellular polysaccharides (EPS) that enable cell aggregation and contribute to protection against drought and osmotic stress (Bashir et al., 2022). According to Kakisina et al. (2023), optimal nutrient availability—specifically nitrogen, phosphorus, and potassium—in biological organic fertilizers enhances photosynthetic efficiency, leading to greater photosynthate allocation for building plant tissues and organs, which ultimately increases biomass accumulation and plant dry weight.

Leaf Area Ratio. Leaf area ratio represents the efficiency of the leaf surface in producing dry matter. Based on Table 8, there is an independent effect of the dose of biofertilizer on the leaf area ratio (LAR). The treatment without biofertilizer produced the highest LAR. The higher the dose of biofertilizer given, the smaller the LAR value obtained. According to Anjarsari et al. (2021), the LAR value is influenced by the area and dry weight of the leaves, which depend on their wet weight, while the wet weight of the plant is related to the transportation of photosynthetic products to the parts that utilize them, such as leaves and stems.

The relatively small leaf area can cause a decrease in LAR compared to leaf dry weight, which can indicate thicker leaves and higher dry matter. Higher leaf weight means that the application of biofertilizer is influenced by the availability of nutrients provided through the leaves and soil. As leaf dry weight increases, the increase in leaf area will decrease. Large leaves can improve the efficiency of light absorption on the leaf surface, but require biomass to increase leaf area (Huang et al. 2019; Sun et al., 2017). Spongy tissue contributes a relatively large amount to leaf thickness and plays a role in storing metabolites and nutrients related to tea quality, such as polyphenols, caffeine, and glucose (Sun et al., 2023).

The microorganisms in the biofertilizer used consist of nitrogen-fixing microorganisms (*Azotobacter* sp. and *Azospirillum* sp.) and phosphate-solubilizing bacteria (*Bacillus* sp. and *Pseudomonas* sp.). These microorganisms help convert substances into a form available to plants, thus indirectly helping to increase the growth of tea plants. Biofertilizers fix nitrogen, dissolve phosphate, secrete growth-promoting substances, reduce the use of chemical fertilizers,

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and improve overall environmental quality (Kumar et al. 2017). According to Figiel et al. (2025), Nitrogenase enzymes can fix and reduce free Nitrogen into a form that plants can absorb, but nitrogenase enzymes are susceptible to oxygen, so nitrogen-fixing bacteria in biofertilizers help bind oxygen and keep the enzyme active. According to Anand et al. (2016), phosphate-solubilizing bacteria promote the dissolution of insoluble phosphate compounds through the secretion of organic acids and the enzymes phosphatase and phytase.

Plant Growth Rate. At the 1st and 2nd plant growth rates, there was no interaction or independent effect of biofertilizer application doses and techniques. This is because the plants were still recovering due to drought. The application of biofertilizers can improve plant growth and development by producing growth hormones such as indole acetic acid (IAA) and cytokinin (Chaudhary et al., 2022).

Table 5 shows the interaction effect of biofertilizer application and fertilization technique at the 4th plant growth rate (PGR). Soil drenching of 30 L ha^{-1} biofertilizer gave the best results, while in foliar application, the dose levels showed relatively similar values. When applied to the soil, microorganisms in biofertilizers can colonize, nitrogen-fixing bacteria (*Azospirillum* sp. and *Azotobacter* sp.) help fix nitrogen, which affects the vegetative growth of plants, and phosphate-solubilizing bacteria help solubilize phosphate, which affects plant metabolism (Mahanty et al., 2017).

Table 6 shows an interaction effect of biofertilizer application doses and techniques on the 5th PGR. At the 6th plucking, applying biofertilizer through the soil at a dose of 15 L ha^{-1} gave the best results, while foliar application requires a dose of 30 L ha^{-1}. This was thought to be due to the presence of previously applied microbes, which still remain in the soil, so the addition of a low dose in the following application was enough to affect the increasing PGR. According to Demir et al. (2023b), when biofertilizers are applied to the soil, the microorganisms colonize the rhizosphere and help improve the absorption efficiency of plant nutrients. According to Ajmal et al. (2018), biofertilizers provide plant nutrients and accelerate microbial activity to provide enough and balanced nutrients for soil and plants.

Table 5. Interaction effect of biofertilizer application techniques and doses on 4th plant growth rate (g m^{-2} day^{-1})

Treatment	Biofertilizer Doses (L ha^{-1})			
	B_1 : 0 L	B_2 : 15 L ha^{-1}	B_3 : 22.5 L ha^{-1}	B_4 : 30 L ha^{-1}
A_1 : Foliar feeding	0.703 a	0.709 a	0.709 a	0.703 b
	A	A	A	A
A_2 : Soil drenching	0.704 a	0.705 a	0.706 a	0.714 a
	B	B	B	A

Note: The mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level. Lowercase letters (a,b) read vertically, comparing the two techniques at the same dose (within a column). Uppercase letters (A, B) read horizontally, compare four doses for the same technique (within a row).

Table 6. Interaction effect of biofertilizer application techniques and doses on the 5th plant growth rate (g m^{-2} day^{-1})

Treatment	Biofertilizer Doses (L ha^{-1})			
	B_1 : 0 L	B_2 : 15 L ha^{-1}	B_3 : 22.5 L ha^{-1}	B_4 : 30 L ha^{-1}
A_1 : Foliar feeding	0.705 a	0.708 a	0.711 a	0.713 a
	B	AB	AB	A
A_2 : Soil drenching	0.707 a	0.715 a	0.707 a	0.702 b
	B	A	B	B

Note: The mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level. Lowercase letters (a,b) read vertically, comparing the two techniques at the same dose (within a column). Uppercase letters (A, B) read horizontally, comparing four doses for the same technique (within a row).

Conclusion

There is a significant interaction effect of biofertilizer application techniques and doses on the shoot dry weight and the growth rate of tea plants. There was an independent effect of biofertilizer dose on leaf area ratio. Soil application at 15 L ha^{-1} and 30 L ha^{-1} resulted in the highest 4th and 5th plant growth rates, and the highest shoot dry weight at the 2nd and 5th harvests. Meanwhile, foliar application at 15 L ha^{-1} and 22.5 L ha^{-1} produced the best shoot dry weight at the 2nd and 6th harvests.

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